Do Changes in Chromosomes Cause Aging?

Minireview

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Aging is a topic that has stimulated curiosity and challenged imaginations since the origins of human consciousness. Perhaps the most interesting new developments in aging center on genes that have been identified in humans and model systems that may speed up or slow down the rate of aging. In humans, one such gene causes the disease Werner's Syndrome, which has several symptoms of premature aging. This disease is caused by recessive mutations in the WRN gene, which was recently cloned (Yu et al., 1996). Werner's individuals display many precocious aging phenotypes as young adults, including cataracts, hair graying and loss, loss of subcutaneous fat, atheroschlorosis, osteoporosis, and carcinomas (Salk et al., 1985). They have an average life span of 45–50 years. However, these individuals also have symptoms that are not observed in normal aging, such as lack of the postadolescent growth spurt and underdevelopment of sexual organs. Other diseases in humans, termed Progerias, cause a rapid onset of aging phenotypes in children. Because these diseases occur sporadically, their underlying basis is obscure, and it is not clear how to identify any genes that may be involved.

Interestingly, the possible functions of WRN and of genes that affect aging in model systems suggest that the primary cause of aging in diverse organisms may be changes that occur in chromosomes. This review will discuss these recent findings in the context of theories of aging and propose several models of how genetic and epigenetic changes in chromosomes might cause aging.

General Models of Aging

Many models for aging fall into two broad categories. The first proposes that aging results from accumulated damage that outstrips repair processes. According to the view of some evolutionary biologists, aging occurs in individuals who are past the age of reproduction, owing to the absence of any evolutionary selection for greater longevity. The second model proposes that aging reflects a genetic program to cull old individuals from the population. It is worth noting that Pacific Salmon undergo rapid senescence after spawning (Finch, 1990), showing that programmed death can occur. These two ideas are not mutually exclusive, in that accumulated damage may be the timing mechanism that triggers a genetic program of aging.

Failure to Repair Damage

The link between damage and aging centers on damage to cellular constituents by oxygen and, possibly, other agents. In this view, oxygen radicals produced as by-products of oxidative phosphorylation cause covalent modifications to macromolecules. In fact, a time-dependent increase in oxidative damage to macromolecules is observed, as exemplified by the accumulation of a lipid–protein cross-linked complex termed lipofuscin (Finch, 1990).

Perhaps the strongest link between oxidative damage and aging is the report that Drosophila strains bearing extra copies of genes encoding both superoxide dismutase and catalase have increased life spans (Orr and Sohal, 1994). Since these enzymes detoxify superoxide anions by converting them into water, this result suggests that oxidative damage may normally limit life span in Drosophila. Buttressing this finding is the *age-1* mutant, which increases life span in Caenorhabditis elegans and also has higher levels of superoxide dismutase (Johnson, 1996). Finally, it is possible that the reason calorically restricted mice, rats, and monkeys live longer than ad libitum–fed controls is that their metabolism is lowered, thereby generating less oxidative damage per unit time.

Genetic Program

Is there evidence for an underlying genetic program for aging, besides rapid senescence in spawning fish? A possible link between life span and stress resistance may bespeak a genetic basis. In C. elegans, a way of coping with starvation is to form Dauer larvae early in development. Whereas the worm has a life span of 2–3 weeks, the Dauer can survive for months in a dormant state. If the Dauer pathway is activated in adults by loss-of-function mutations in *daf-2*, the worms live 2-fold longer than controls (Kenyon et al., 1993). In the budding yeast Saccharomyces cerevisiae, different strains within the sample studied display different sensitivities to death due to starvation. Starvation-resistant strains also turn out to have the longest life spans (Kennedy et al., 1995). In this organism, life span is defined by the number of times a mother cell divides before senescing and is measured by microscopic manipulation of mothers away from their smaller daughters.

Is There a Universal Mechanism of Aging?

The study of aging in model systems, such as Drosophila, C. elegans, and S. cerevisiae would be particularly justified if there were a universal mechanism of aging affecting these organisms, as well as mammals. A universal mechanism would be consistent with a model for aging as the sum of effects on individual cells, rather than as a process acting at the level of the organism.

There are several suggestions that aging can act at the cellular level. First, dysfunction in many organs with age is due to a loss of function of cells. This includes the loss of neurons in the brain, leading to a reduction in cognition; loss of the subcutaneous fat cell layer, leading to a loss of suppleness of the skin; and loss of melanin production in hair follicle melanocytes, leading to graying of the hair.

Second, primary explants of animal cells can divide only a limited number of times in culture before becoming postmitotic (Hayflick, 1965; Campisi, 1996). It has been proposed that this replicative senescence is mechanistically related to aging in the animal. Consistent with this view, the replicative capacity of cells explanted from a variety of mammals is roughly proportional to the life spans of the animals.

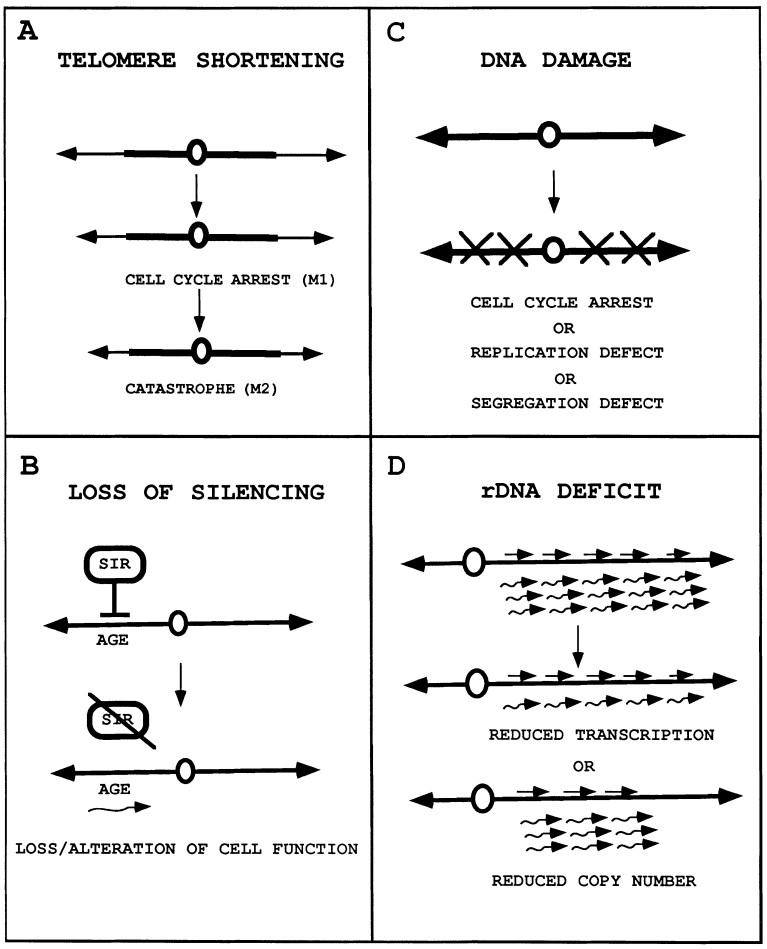

Figure 1. Structural or Functional Changes in Chromosomes That May Cause Aging

(A) Telomere shortening in cultured human cells leads to cell cycle arrest (M1). If this is overridden by the action of T antigen, further shortening can lead to catastrophe (M2) due to deletion of essential genes (Greider and Harley, 1996).

(B) Silencing machinery that renders chromosomal domains transcriptionally inert may become inactive with age. The resulting alteration in gene expression in cells may cause a loss of cell function and aging.

(C) DNA damage accumulates with age, leading to cell cycle arrest, or a defect in replication, or chromosome segregation. The identification of the *WRN* gene as a putative DNA helicase is superficially consistent with this view.

(D) Changes in expression of rRNA occur with age due to a reduced ability to transcribe rDNA repeats or a reduction in the number of repeats due to recombination. This model arises from the link between the yeast *WRN* homolog and topoisomerases 2 and 3, which function in rDNA maintenance and transcription, respectively.

Third, WRN is homologous to a family of DNA helicases, including recQ of Escherichia coli (Yu et al., 1996). This connection between Werner's and DNA metabolism is consistent with a view that aging is a cellular-autonomous process. More specifically, this finding is consistent with a connection between aging and changes in chromosomes, as discussed below.

Are system-wide processes also important? An old idea is that aging is due to mechanical wear and tear. In fact, there is evidence that elephants and hippopotami can die of starvation because their teeth have worn down (Finch, 1990). While this theory is obviously simplistic, it is possible that some changes in people *are* due to wear and tear and would occur even if all other aging mechanisms could be halted. These might include, for example, atherosclerosis due to mechanical stress in veins and arteries and lining and wrinkling of the skin due to repetitive movements and exposure to the elements.

Beyond this simple idea of mechanical wear, there is evidence of system-wide deficiencies that occur with age in immunological and endocrinological systems. This correlative data has led to unproven, simplified claims that aging can be reversed by supplementation with hormones that decline with age, such as melatonin or growth hormone.

Possible Molecular Mechanisms of Aging

Even at this primitive stage in the identification of genes affecting life span, it is tempting to speculate on possible molecular mechanisms of aging. I propose four such mechanisms below, which center on the possibility that changes in chromosomes may cause aging.

Telomere Shortening

There is evidence that telomeres shorten in the soma of people, with time; and in cultured cells, as they divide due to the lack of telomerase in these cells. In cultured human cells that divide toward their limit, telomeres shorten, resulting in G1 arrest and senescence (de-Lange, 1994; Greider and Harley, 1996; Wright and Shay, 1996; Figure 1A). This block, termed M1, can be overridden by transforming genes such as T antigen of SV40. However, telomeres continue to shorten, and cell death results in a step termed M2, possibly due to the loss of essential genes at the ends of chromosomes or a loss of mitotic stability of shortened chromosomes. Rare clones that grow out are immortalized and have acquired telomerase activity and stabilized telomeres. Thus, a model that relates telomere shortening to aging rests solely on correlative data in humans.

A limitation of this model is that it is difficult to generalize it to other organisms. In mice, telomeres are very long, and telomerase activity is present in somatic cells. In yeast, telomeres do not shorten in old cells. It is thus

difficult to imagine telomere shortening as a cause of aging in these organisms. To begin to approach the question of causality, it will be necessary to provide a constant level of telomerase activity in cultured cells using cloned genes and to determine whether they display an extended division capacity.

Loss of Silencing

In yeast, a loss of silencing has been causally related to aging. A mutation in *SIR4* extends life span by 50% (Kennedy et al., 1995). *SIR4*, along with *SIR2* and *SIR3*, silences *HML* and *HMR*, containing silent mating-type information, and also genes positioned at telomeres. The *SIR4* mutation that extends life span behaves like a *sir4* null mutation at *HML*, *HMR*, and telomeres; i.e., silencing is abolished. However, a null allele of *SIR4* actually *shortens* life span. This means that the life span extension of the mutant *SIR4* is due to a gain of function. It has thus been proposed that the *SIR4* mutation redirects the SIR complex away from telomeres and *HM* loci to a novel locus (*AGE*) to delay aging. In this model, the *AGE* locus is silenced in young cells and turned on in old cells (Figure 1B) to cause aging. Consistent with this model, silencing by the SIR complex at *HML* and *HMR* is, in fact, lost in old cells, resulting in the activation of *HML* and *HMR* and thereby causing sterility (Smeal et al., 1996). In higher organisms, the analog of silenced regions in the yeast genome consists of inert regions of chromosomes identified cytogenetically and termed heterochromatin. While there is not strong evidence showing that maintenance of heterochromatin breaks down with age, one study found that a gene on the silenced X chromosome was reactivated in old mice (Wareham et al., 1987).

It may be relevant to consider the clock (clk^-) mutants of C. elegans, which lengthen the period of many temporal processes, including development, cell cycle, and also the periods of rhythmic behaviors in adults, including pharyngeal pumping, swimming, and defecation (Wong et al., 1995). Most strikingly, *clk* mutations lengthen life span. These mutants thus behave as though they have altered the setting of a clock that times many processes, including life span (Kenyon, 1996). Interestingly, *clk* mutants all exhibit a maternal effect. This means that progeny with a clk^-/clk^- genotype that are derived from a homozygous mutant clk^-/clk^- hermaphrodite display the Clk^- mutant phenotypes, but progeny with the clk^-/clk^- genotype that are derived from a clk^-/clk^+ hermaphrodite display a wild-type phenotype. Such a pattern of inheritance implies that the clk^+ gene exerts its effect in gametogenesis or very early in development, and its absence at these times exerts lifelong effects, including an extended life span.

How might *clk* genes exert an effect in gametogenesis that lasts into adulthood and affects life span? One possibility is that the *clk* genes exert their effects on some aspect of chromosome structure or function. A specific model is that *clk* genes affect silencing of regions of chromosomes. Once silenced chromatin is established, it can be stable through many rounds of cell division. It is possible that in gametes derived from clk^-/clk^- parents, certain regions of chromosomes are silenced to a greater degree than in gametes from parents with a clk^+ gene. This state could persist for the lifetime of the animal and influence the periodicity of rhythmic behaviors and also affect life span. Of course, other possible models involving chromosomes, for example control of telomere length by *clk* genes, are also possible.

Accumulated DNA Damage

The identification of *WRN* as a DNA helicase fortified an old hypothesis that DNA damage or mutations could be a cause of aging. A failure in repair in Werner's individuals would thus lead to cellular defects (Figure 1C) and the phenotype of premature aging. Consistent with this hypothesis, Werner's cells are reported to have a higher mutation rate than normal fibroblasts (Salk et al., 1985). However, a DNA-repair deficit has not been demonstrated in Werner's cells. Moreover, another DNA helicase of this class is the gene mutated in Bloom's syndrome (Ellis et al., 1995), and the symptoms of this disease are not reminiscent of premature aging but include cancers and sunlight sensitivity. The idea that aging phenotypes in Werner's Syndrome result from accumulated DNA damage or mutations is thus problematic.

WRN and the Blooms gene have extensive amino- and carboxylterminal regions that are outside the helicase domain and are not homologous. It is possible that the *WRN* helicase is targeted for a specific function by these sequences outside the helicase domain. In fact, the four mutations in *WRN* described were all chain-terminating mutations carboxyl to the helicase domain of the protein (Yu et al., 1996). Clues about the specificity of the *WRN* helicase may come from studying *WRN* homologs in model systems (see below).

Defect in rDNA Transcription or Maintenance

The *WRN* helicase domain is found in the sequence of the yeast gene, *SGS1* (Gangloff et al., 1994; Watt et al., 1995). This gene was identified by genetic and two-hybrid interactions with yeast *TOP2* and *TOP3*, suggesting that the activities of the topoisomerases and the DNA helicase are coupled. What are possible functions of a TOP–SGS1 complex? Two possibilities relate to the rDNA locus. First, mutations in *top3* or *sgs1* increase recombination within the hundreds of copies of rDNA that are tandemly repeated in the genome (Gangloff et al., 1994). Intriguingly, mutations in *SIR2* also increase recombination in the rDNA (Gottlieb and Esposito, 1989), suggesting that the *SIR4* mutation, described above, may lengthen life span not by repressing transcription of an *AGE* locus but by preventing recombination at the rDNA. Thus, hyperrecombination in rDNA could lead to a loss of rDNA copies in aging yeast cells and in Werner's individuals.

Second, TOP–SGS1 may influence transcription of rDNA. In *top1 top2*^{ts} strains that are shifted to the nonpermissive temperature, rDNA transcription stops, while transcription of mRNA continues (Brill et al., 1987). *WRN*, therefore, may play a role in rDNA transcription. A specific defect in rDNA transcription or maintenance (Figure 1D) could result in fewer ribosomes, decreased protein synthetic capacity, and aging-specific phenotypes. However, *TOP2* is also involved in DNA replication, and the possibility that the *WRN* function relates to replication cannot be excluded.

Conclusion

It is clearly too early to know with certainty what molecular events cause aging. An exciting possibility based on

recent findings is that changes in chromosomal structure or function are a key determinant of aging. The methodology providing this new edge in aging research is the identification of specific genes that control the rate of aging in S. cerevisiae, C. elegans, and humans. It would perhaps be appropriate that chromosomes, which orchestrate the genesis, development, and maturation of organisms, also direct the final chapter in the life cycle.

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